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National Toxicology Program

The NTP HTS Initiative: An Update

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To meet the challenge of 21st century toxicology, the NTP Roadmap includes a major new initiative to develop a high throughput screening (HTS) program with 3 main goals:

- Identify mechanisms of action for further investigation
- Develop predictive models for *in vivo* biological response
- Prioritize substances for further in-depth toxicological evaluation



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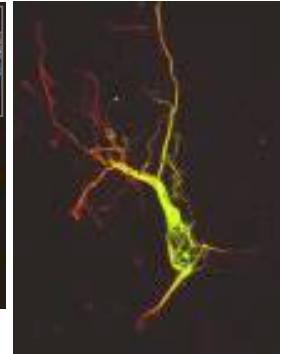
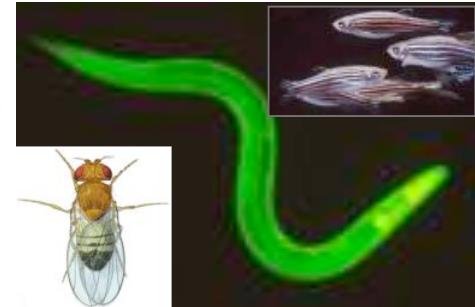
What can be screened?



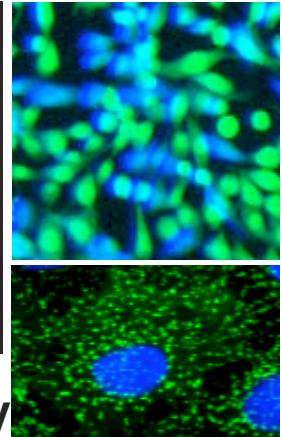
10's/year



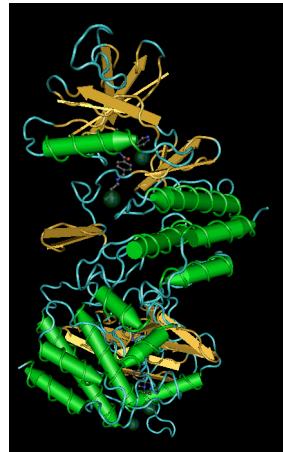
100's/year



10,000's/day



100,000's/day



1-3/year

High Throughput
Molecular mechanism

Immediate Human Relevance



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NIH Molecular Libraries Initiative

<http://nihroadmap.nih.gov/molecularlibraries/>

- The MLI is using HTS methods to identify small molecules that can be optimized as chemical probes to study the functions of genes, cells, and biochemical pathways.
- In mid-2005, NTP became a formal participant in the MLI by establishing a collaboration with Drs. Chris Austin and Jim Inglese of the NIH Chemical Genomics Center (NCGC) (<http://www.ncgc.nih.gov/>)
- Thus, the NTP has the opportunity to link data generated from HTS assays for biological activity to data produced by the NTP's toxicology testing program.



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The NTP “1408”

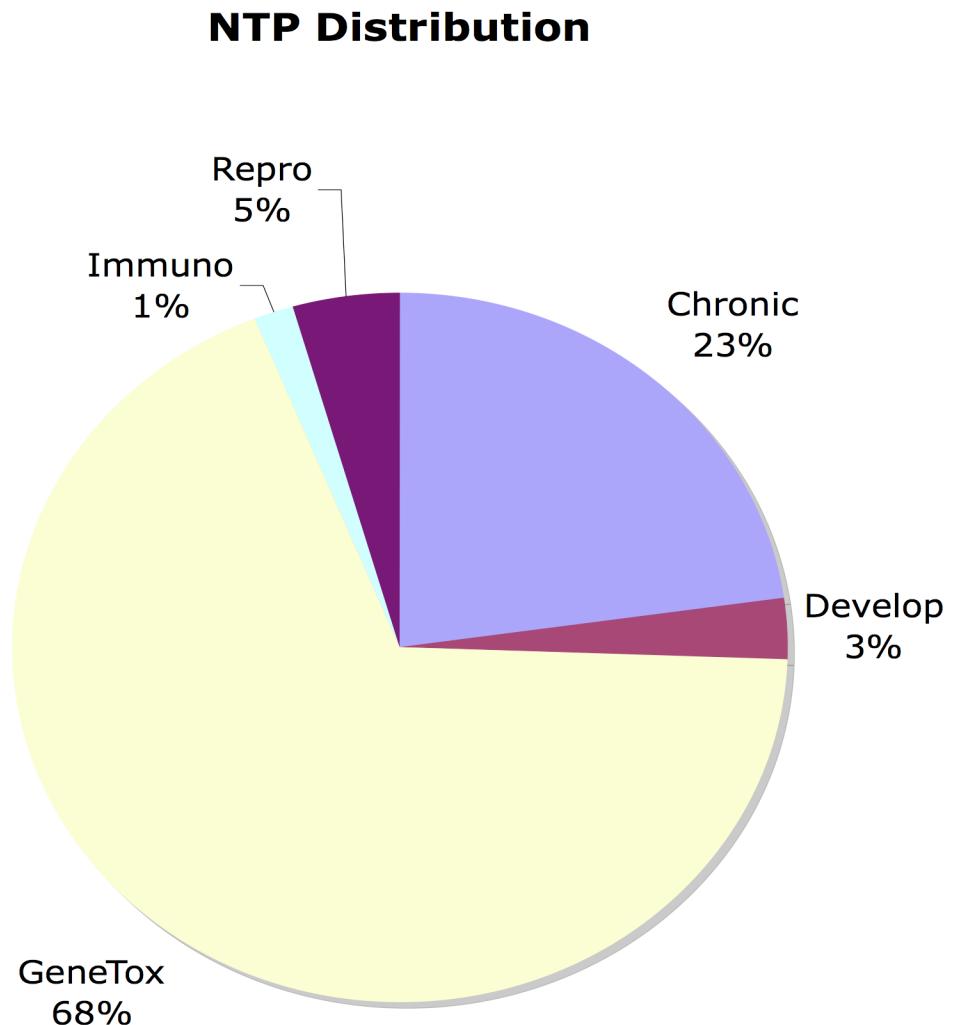
- Provided as 10 mM solutions dissolved in DMSO
- 55 Duplicates
- Includes nearly every chemical class
- Molecular weights range from ~100 to ~400
- All have pre-existing toxicity data
 - 1206 with NTP test data
 - 147 are reference substances identified by ICCVAM for the validation of alternative *in vitro* test methods (e.g., dermal corrosion, acute toxicity, endocrine activity).
- Includes solvents, fire retardants, preservatives, flavoring agents, plasticizers, therapeutic agents, inorganic and organic pollutants, drinking water disinfection byproducts, pesticides, and natural products
- In negotiation with MLI to provide compounds as a specialty set for use by all Centers



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The 1206 -
distribution
among individual
NTP assays





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The 1206 - distribution among NTP assays, by number of assays tested

Studies	Count
none	12
Chronic	12
Chronic & Devel	1
Chronic & GeneTox	305
Chronic & Devel & GeneTox	11
Chronic & Immuno & GeneTox	6
Chronic & GeneTox & Repro	34
Chronic & Devel & GeneTox & Immuno	1
Chronic & Devel & GeneTox & Repro	12
Chronic & GeneTox & Immuno & Repro	5
Chronic & Devel & GeneTox & Immuno & Repro	2
Devel	2
Devel & GeneTox	10
Devel & Repro	1
Devel & GeneTox & Repro	4
GeneTox	760
GeneTox & Immuno	7
GeneTox & Repro	9
GeneTox & Immuno & Repro	1
Immuno	2
Immuno & Repro	1
Repro	8
Total	1206



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The Next 1408

- IRIS, Carcinogenic Potency, and HPV databases merged and duplicates subtracted
- Subtracted first 1408
- Subtracted MW<80; MW>700
- Added on-plate duplicates from first 1408
- Solicited suggestions from NIEHS community
- A goal is to include structurally-related compounds that cover the complete activity range
- Focus on compounds tested for a specific toxicological endpoint (e.g., immunotox, cancer)



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HTS assays supplied to the NCGC

- **Apoptosis Assays**
 - Caspase-Glo® 3/7 Assay
 - Caspase-Glo® 9 Assay
 - Caspase-Glo® 8 Assay
- **Cytotoxicity Assays**
 - CellTiter-Glo® Luminescent Cell Viability Assay (measures ATP levels)
 - Cytotox-ONE™ Homogeneous Membrane Integrity Assay (measures release of lactate dehydrogenase from membrane-damaged cells)
- **P-glycoprotein (Pgp) ATPase Assay (aka MDR1 or ABCB1)**
 - Pgp-Glo™ Assay



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Cell lines being screened at the NCGC against NTP assays

Human cell lines

HEK 293	Transformed kidney
HepG2	Hepatoma
SH-SY5Y	Neuroblastoma
Jurkat	Acute T-cell leukemia
BJ	Foreskin fibroblasts
HUV-EC-C	Umbilical vein vascular endothelium
MRC-5	Lung fibroblasts
SK-N-SH**	Neuroblastoma (will not be included in future screens)

Rodent cell lines

Primary Renal Proximal tubule cells – rat	
H-4-II-E	Liver carcinoma – rat
N2a	Neuroblastoma – mouse
Buffy coat	Lymphocytes – rat
NIH 3T3	Embryonic fibroblasts -- mouse



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Summary of NCGC Testing Conducted to Date and PubChem Status

Human cell lines	CellTiter-Glo	PubChem	Caspase-3	LDH
Hek 293	Mar-06	✓	Jun-06	
HepG2	Jan-06	✓	Jun-06	Assay development
SH-SY5Y	Apr-06		Aug-06	
Jurkat	Jan-06	✓	Jun-06	
BJ (skin fibroblasts)	Mar-06	✓		
HUV-EC-C	Jul-06		Jul-06	
MRC-5 (lung fibroblasts)	Mar-06	✓		
SK-N-SH*	Mar-06	✓		
Rodent cell lines	CellTiter-Glo	PubChem	Caspase-3	LDH
Renal proximal tubule cells (rat)	Sep-06 **		Sep-06 **	Assay developed
H-4-II-E (rat)	Jun-06		Jun-06	
N2a (mouse)	Jun-06		Aug-06	
Buffy coat (rat)				
NIH 3T3 (mouse)	Jun-06		Jul-06	

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Other NCGC HTS Assays

Project Name	Disease application (if any)
Acrosome reaction GFP	
Anthrax LF BLA	Anthrax
ATR Activation	Ataxia telangiectasia (OMIM 607585)
β -AdrR PCA bifurcated GFP	
β -lactamase (AmpC)	
β -glucocerebrosidase FI	Gaucher disease (OMIM 230800)
β -Thal mRNA splicing GFP	Beta-thalassemia
Caspase 3	
Cell signaling AP-1-BLA	
Cell signaling CRE-BLA	
Cell signaling HRE-BLA	
Cell signaling M1 NTR	
Cell signaling NFAT-BLA	
Cell signaling SIE-BLA	
Cell Translocation GR-EFC	
Cell Translocation GR-GFP	
Cell Translocation p65 HaloTag	
Cellular Toxicity (ATP level)	
Cellular Toxicity (LDH level)	
cLANA	HSV
Cpd aggregation FRET-1 (AggFRET)	
DNA damage GFP-x gene	
Drosophila Fat cell GFP	
ER/GR Translocation	
Fluor-DNA displacement-1	
Fluorescent Profiling-1	
GPVI Luciferase	
HIV Nucleocapsid FP	HIV
Hsp90 co-chaperone interaction	
Huntingtin PC12 cell toxicity	Huntington's Disease (OMIM 143100)
I κ B α Cell sensor Dual Luc	Rare lymphomas
JNK ALPHAScreen	

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Other NCGC HTS Assays

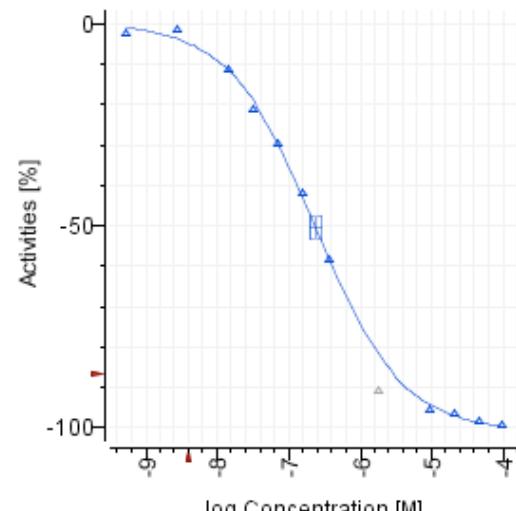
Project Name	Disease application (if any)
Locus Derepression Assay-1 GFP	
Luciferase profiling	
Malarial PSAC	Malaria
Multi-protein DNA Replication System	
O-Glc NAc Transferase	
Opsin trafficking ALPHA	Retinitis pigmentosa (OMIM 180380)
orphan GPCR -ADHD	ADHD
Oxidoreductase HADH2	
Oxidoreductase DCXR	
Oxidoreductase HSD17b4	
Oxidoreductase retSDR3	
Oxidoreductase SPR	
P450 CYP1A2, Luc	
P450 CYP2C9, Luc	
P450 CYP2C19, Luc	
P450 CYP2D6, Luc	
P450 CYP3A4, Luc	
Pantothenate Kinase	Tuberculosis
Peroxiredoxins (Tgr-Trx-Prx)	Schistosomiasis
PI5K4Pbeta	Diabetes
Progeria mRNA splicing GFP/RFP	Progeria (OMIM 176670)
Proteosome ubiquitin-GFP	Various
PyruvateKinase Luc	Hemolytic anemia (OMIM 266200)
RAS-RAF PCA bifurcated GFP	
Sialic aciduria	Sialuria (OMIM 269921)
SMA Cellular promoter act BLA	Spinal Muscular Atrophy (OMIM 253300)
Tau polymerization	Alzheimer, Frontotemporal dementia (OMIM 600274)
TF assay-cancer	Cancer
TPO Luciferase	Thrombocytopenia
Ubiquitin Pathway	Various
YjeE FP	



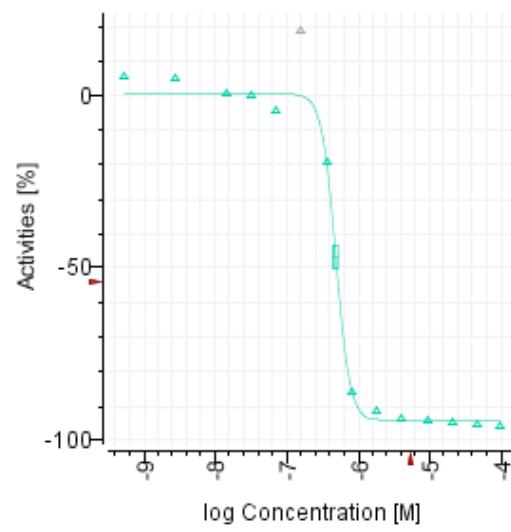
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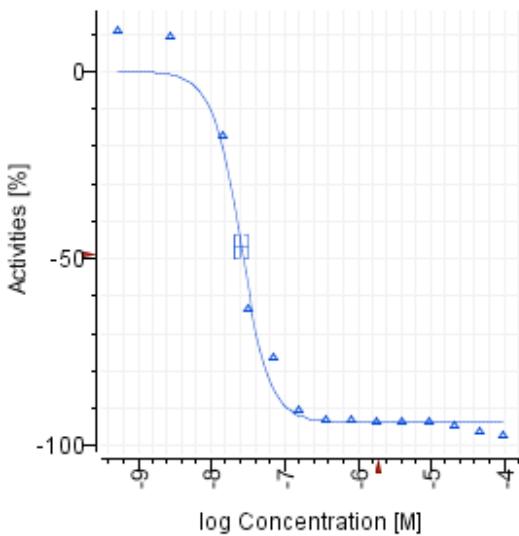
Cell viability dose response



AB08002816-01



AB08002816-1



AB08002816-1

NCGC provides normalized and corrected values and computes AC50

Each dilution is assayed in a separate plate, with in-plate controls

Doxorubicin and Tomaxifen dose response in duplicate

Column of Basal, column of 100 μ M Tomaxifen is used to define 0% and 100% “death”

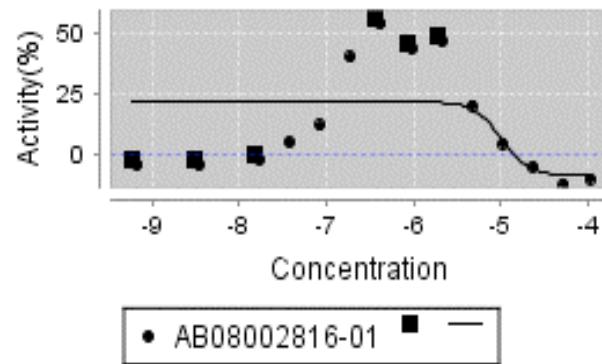


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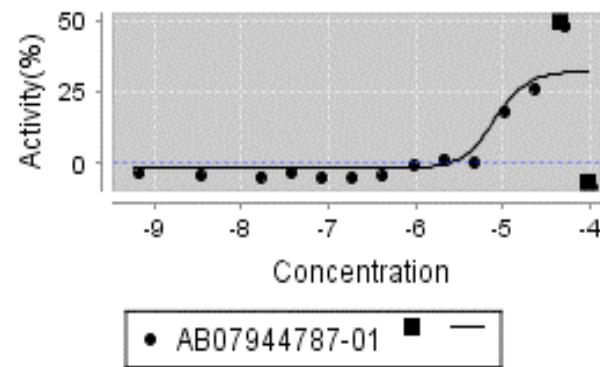
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Caspase assay response

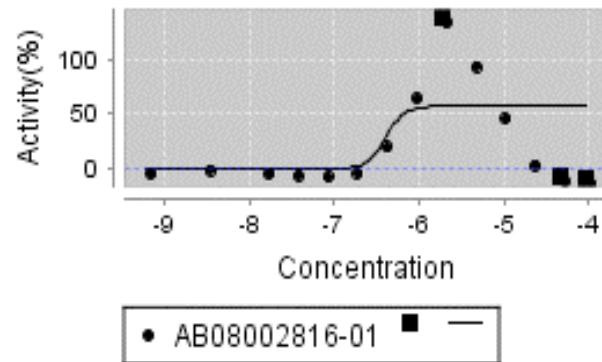
Response



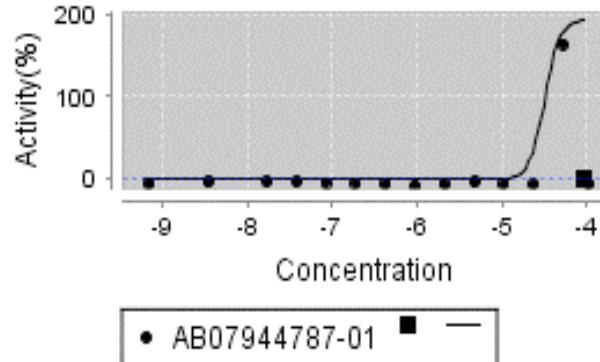
Response



Response



Response





@ AID 427 – PubChem BioAssay Summary

Address: <http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=427> go

Back Forward Stop Refresh Home AutoFill Print Mail

Live Home Page Apple Apple Support Apple Store .Mac Mac OS X Microsoft MacTopia Office for Macintosh MSN

NCBI PubChem National Library of Medicine NLM

HOME SEARCH SITE MAP PubMed Entrez Structure GenBank PubChem Help

BioAssay Summary

AID: 427 Name: Cell Viability - Hek293 Data Source: NCGC

Test Results: Show Select Plot

Links Description Protocol Comment Definitions

Links:

Compounds: All: 1335 Active: 74 Inactive: 1180 Inconclusive: 86

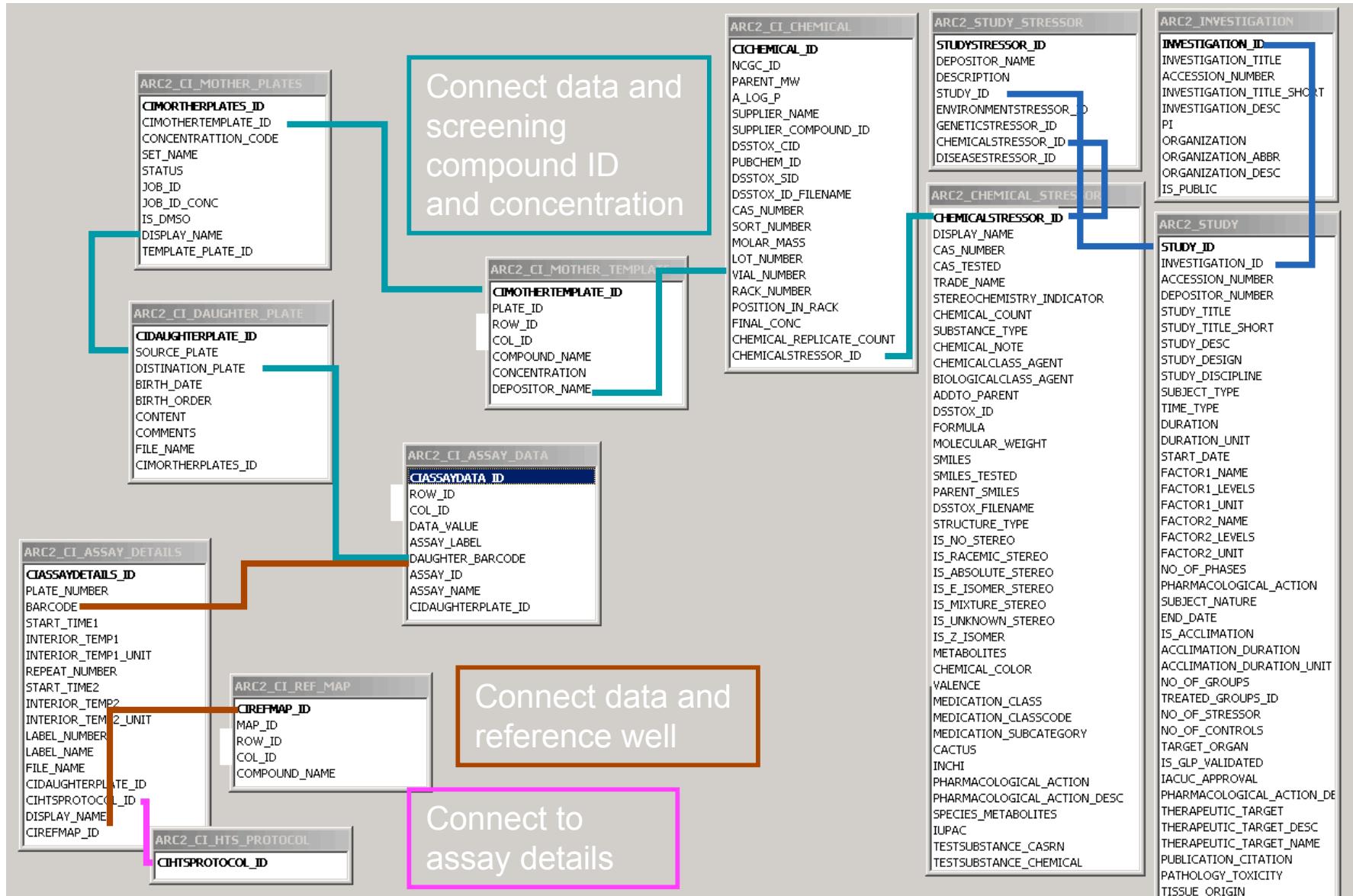
Substances: All: 1408 Active: 80 Inactive: 1241 Inconclusive: 87

Taxonomy: 1 Link

Description:

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Screening Centers Network [MLSCN]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]

Internet zone

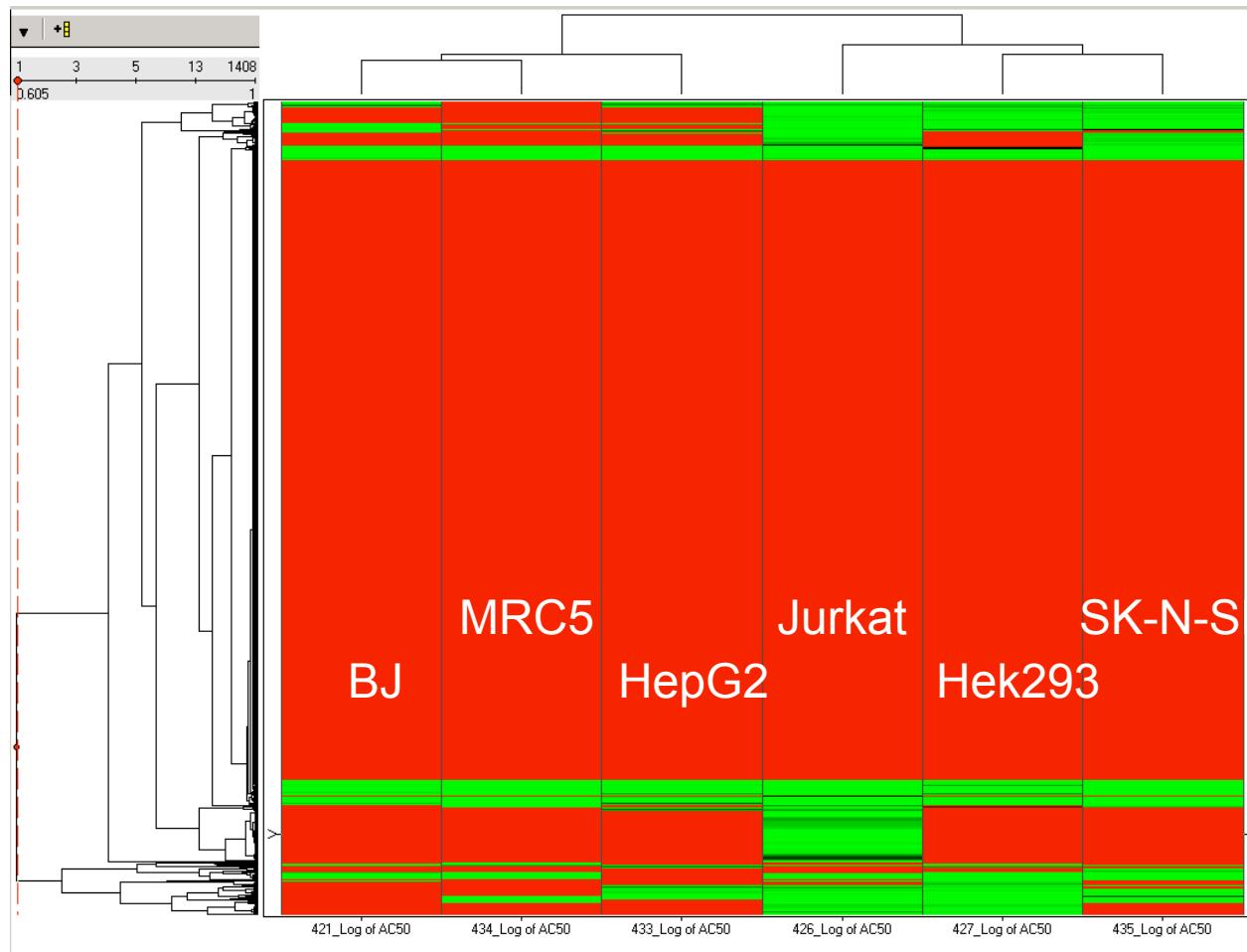




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Heatmap: log(AC50) organized by correlation



435 – SK-N-SH
434 – MRC5
433 – HepG2
427 – Hek293
426 – Jurkat
421 -- BJ

Clustering method: Complete linkage (maximum)
Similarity measure: Cosine correlation
Ordering function: Input rank

Colors:
Default
-4 -2

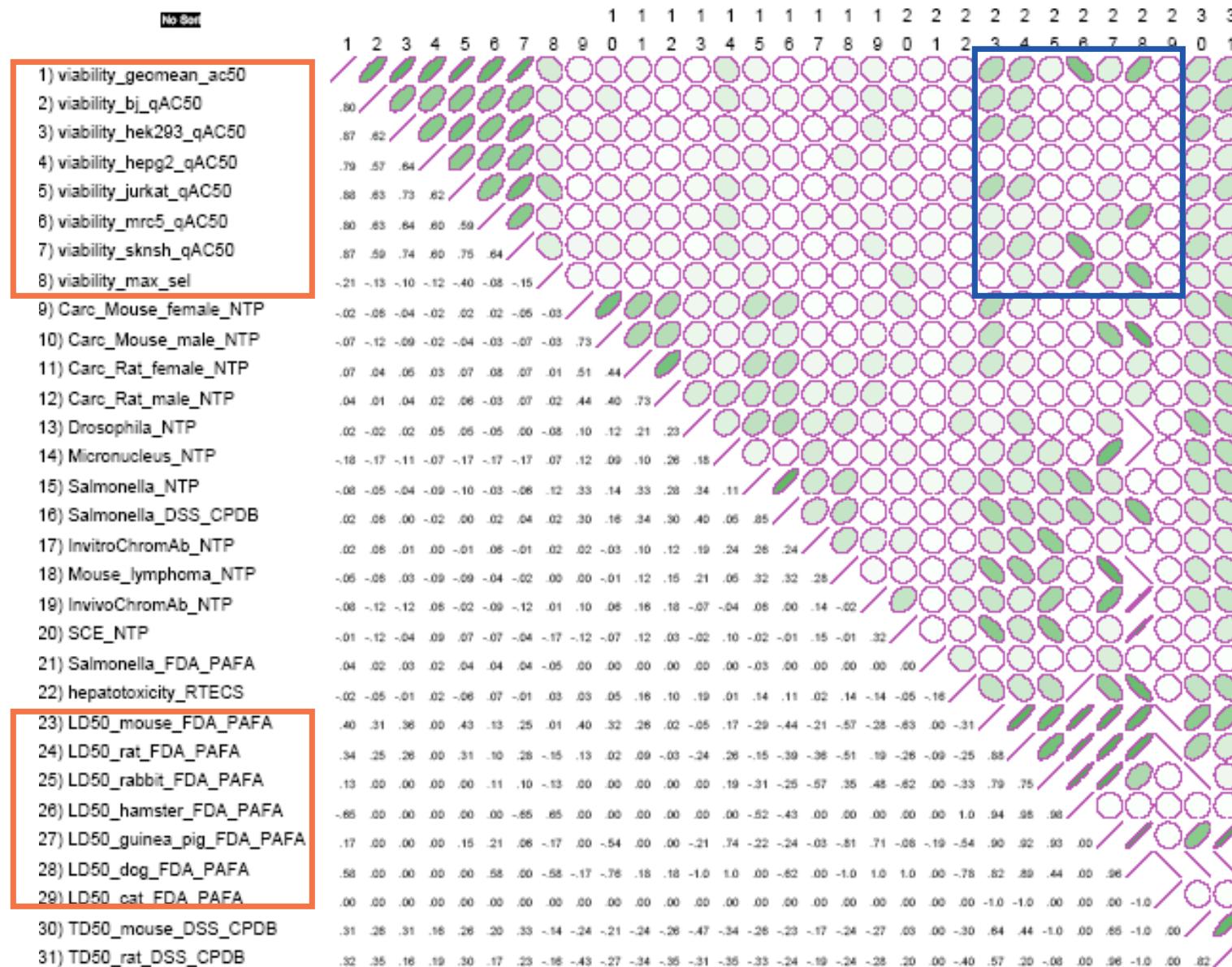
remove the ~ 1000 compounds that were not active (AC50 = -2)



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Comparison of *In Vitro* and *In Vivo* Studies

Comparison of *In Vitro* and *In Vivo* Studies

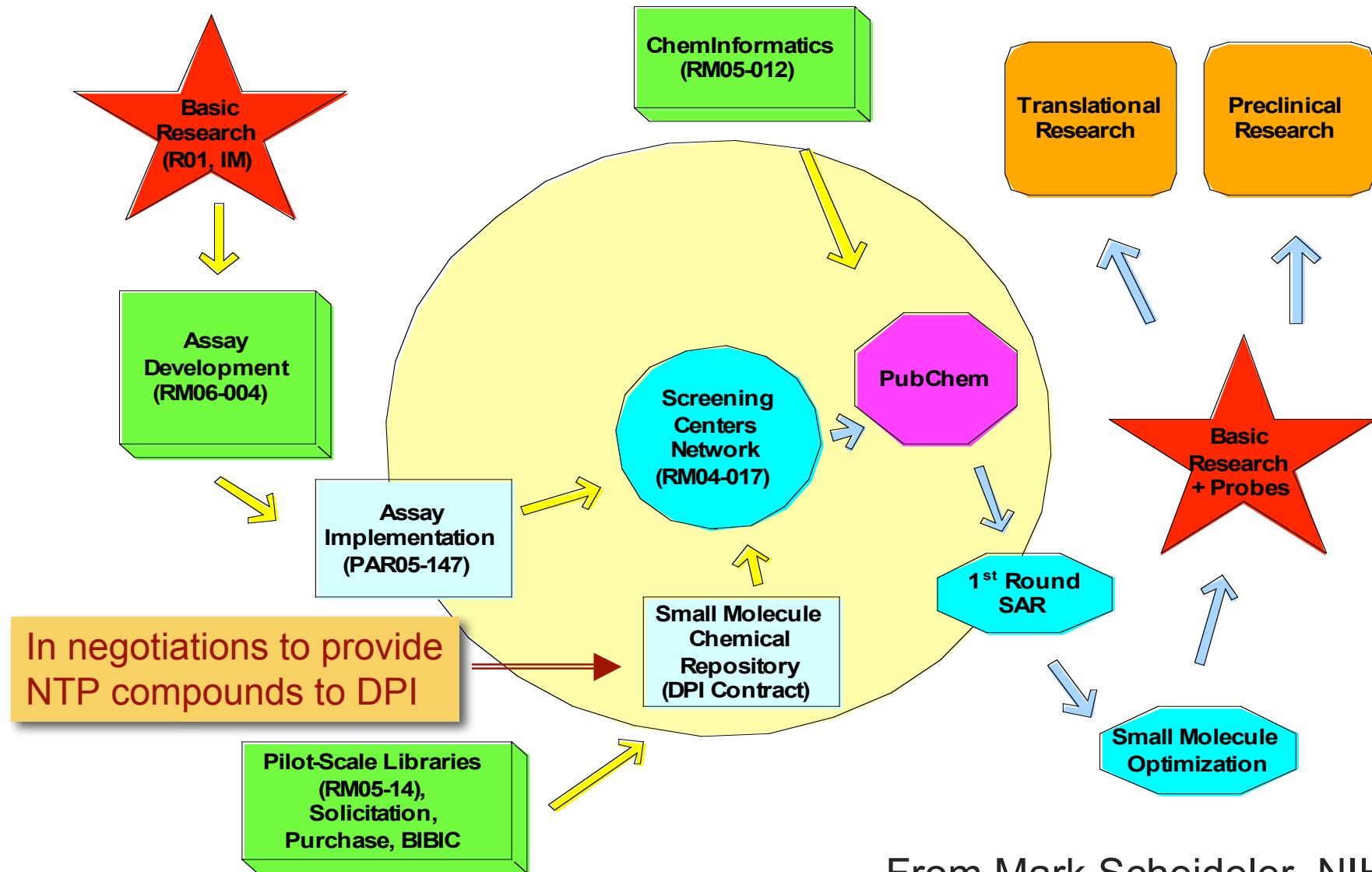




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Roadmap Molecular Libraries Initiative (<http://nihroadmap.nih.gov/molecularlibraries/>)



From Mark Scheideler, NIH



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Molecular Assay Targets

■ Enzyme

■ Channel or Transporter

■ Protein Folding

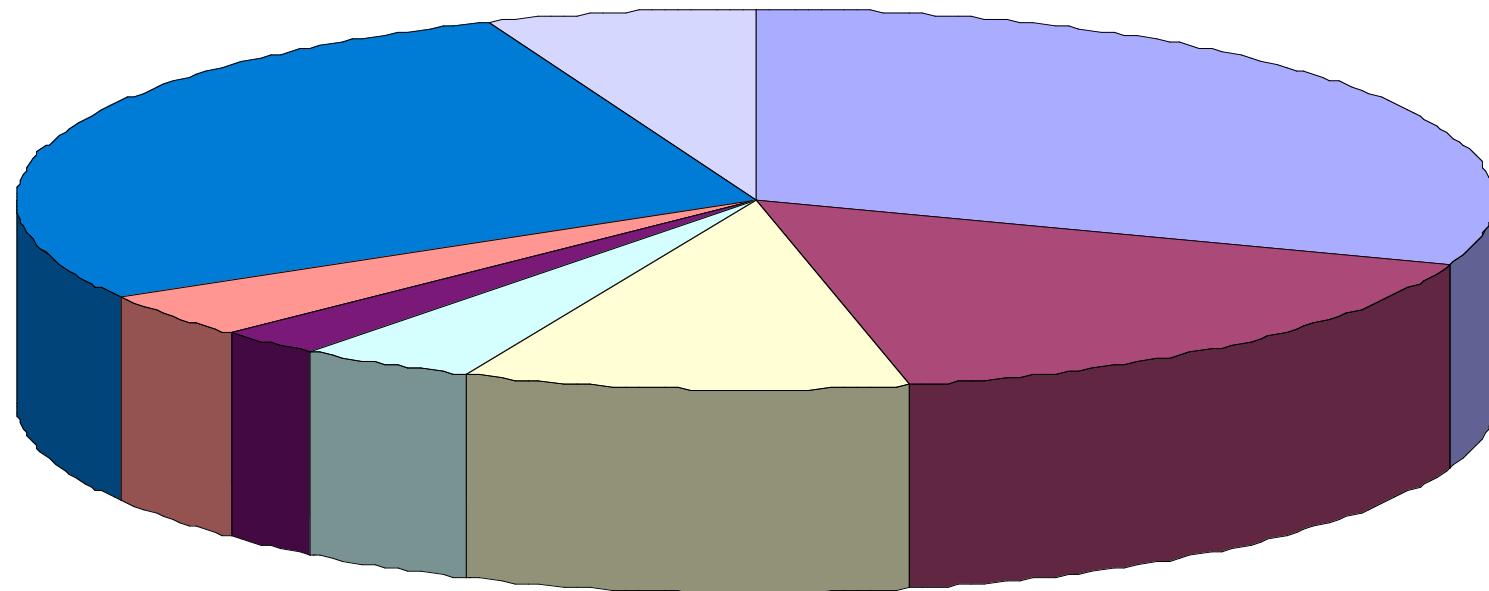
■ Protein-Protein Interaction

■ Receptor

■ Protein Synthesis-Modification

■ Translocation-Secretion

■ Transcription-Splicing





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NTP-EPA Collaboration on HTS Assays

- In Dec. 2005, a collaboration was established between the NTP HTS Faculty and the EPA Chemical Prioritization Community of Practice (CPCP) to jointly evaluate HTS assays and other model systems for their use in toxicological investigations and in chemical prioritization.
- In Jan, 2006, joint NTP/EPA subcommittees were established to address specific topics related to HTS
 - Toxicity targets and bioactivity assays
 - Co-Chairs: Kristine Witt (NIEHS) and Keith Houck (EPA)
 - Chemical selection
 - Co-Chairs: Cynthia Smith (NIEHS) and David Dix (EPA)
 - Informatics
 - Co-Chairs: Jennifer Fostel (NIEHS) and Ann Richard (EPA)



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- **Expand the number of compounds**
 - Structurally-related compounds with a range of toxicities
 - Parent and metabolites
 - Mixtures
- **Expand the number of HTS assays**
 - P450s (CYP1A2, CYP3A4, CYP2D6)
 - Critical Pathways: AP1, STAT, NFAT, HRE, NFkB, nuclear hormone signaling
 - NCGC assays
 - MLI assays
 - NTP selected assays
- **Expand the number of cell types**
 - Primary cells
 - Different species
- **Expand chem- and bio-informatics capabilities**